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**TRANSMITTAL LETTER TO THE UNITED STATES  
DESIGNATED/ELECTED OFFICE (DO/EO/US)  
CONCERNING A FILING UNDER 35 U.S.C. 371**

1871-129

U.S. Application No. (if known, see 37 CFR 1.5)

**09/509196**

**INTERNATIONAL APPLICATION NO.**  
PCT/AU98/00795

**INTERNATIONAL FILING DATE**  
23 September 1998

**PRIORITY DATE CLAIMED**  
23 September 1997

**TITLE OF INVENTION**

A Potential Effector for the Grb7 Family of Signalling Proteins

**APPLICANT(S) FOR DO/EO/US**

Roger John DALY, Robert Lyndsay SUTHERLAND

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
  - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☒ has been transmitted by the International Bureau.
  - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
  - a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
  - b. ☐ have been transmitted by the International Bureau.
  - c. ☐ have not been made; however, the time limit for making such amendments has **NOT** expired.
  - d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

**ITEMS 11. TO 16. below concern other document(s) or information included:**

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A **FIRST** preliminary amendment.  
☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:
  - Copy of published application 99/15647
  - Copy of International Preliminary Examination Report
  - Sequence Listing in computer readable format

[illegible]

1871-129  
EE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)	
	)	
Roger J. DALY et al.	)	Filing Under 35 USC 371
	)	International Application
Serial No.	)	No. PCT/AU98/00795
	)	Filed: 23 September 1998
Filed:	)	
	)	
For: A POTENTIAL EFFECTOR FOR	)	
THE GRB7 FAMILY OF	)	
SIGNALLING PROTEINS	)	

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents  
Washington, D.C. 20231

Dear Sir:

Prior to calculating the filing fee for the above-referenced patent application, please enter the following amendments:

In the Claims:

In claim 5, line 2, please delete "any one of the preceding claims" and insert therefor --claim 1--.

In claim 7, line 2, please delete "or 6".

In claim 8, line 2, please delete "any one of claims 1 to 4" and insert therefor --claim 1--.

In claim 11, line 2, please delete "or 9".

In claim 12, line 4, please delete "any one of claims 1 to 4" and insert therefor --claim 1--.

In claim 15, line 3, please delete "or 13".

Please add the following new claims:

16. An antibody or fragment thereof which specifically binds to a protein according to claim 9.

17. A method of detecting in a sample the presence of an effector protein for the Grb7 family of proteins, the method comprising reacting the sample with an antibody or fragment thereof according to claim 16.

18. A method of detecting in a sample the presence of mRNA encoding an effector protein for the Grb7 family of proteins, the method comprising reacting the sample with an oligonucleotide probe of claim 13.

REMARKS

The amendments set forth above are made to simplify the claim dependencies. No new matter is introduced into the application by means of these amendments.

Respectfully submitted,

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**A POTENTIAL EFFECTOR FOR THE GRB7 FAMILY OF SIGNALLING  
PROTEINS**

**Field of the Invention:**

5

The present invention relates to a novel polynucleotide molecule encoding a candidate effector protein for the Grb7 family of signalling proteins. Detection of the encoded protein in a tissue sample should provide a useful tumour marker and/or prognostic indicator. Furthermore, antagonism of the interaction between Grb7 family members and the encoded protein should provide a novel treatment strategy for human diseases exhibiting aberrant receptor tyrosine kinase (RTK) signalling (e.g. cancer).

10  
15 **Background of the Invention**

RTKs play a major role in the regulation of cellular growth, differentiation, motility and metabolism by converting an extracellular signal in the form of the binding of a specific hormone or growth factor to the activation of specific signalling pathways and hence modes of intracellular communication (Schlessinger and Ullrich, *Neuron* 9, 383-391, 1992). Activation of RTKs results in both autophosphorylation of the receptor and the phosphorylation of downstream targets on tyrosine residues. It has become evident over the last decade that key elements in receptor-substrate and other protein-protein interactions in RTK signalling are src homology (SH)2 domains. SH2 domains are conserved modules of approximately 100 amino acids found in a wide variety of signalling molecules which bind to short tyrosine-phosphorylated peptide sequences. The specificity of interaction is determined both by the nature of the amino acids flanking the phosphotyrosine residue in the target peptide and residues in the SH2 domain which interact with these sites (Pawson, *Nature* 373, 573-580, 1995).

SH2-domain containing proteins can be divided into two classes: those which possess a catalytic function (e.g. the cytoplasmic tyrosine kinase c-src and the tyrosine phosphatase SH-PTP2) and those which consist entirely of non-catalytic protein domains (eg Grb2), the adaptor sub-class. The function of the latter class is to link separate catalytic subunits to a tyrosine-

phosphorylated receptor or signalling intermediate, and other non-catalytic protein modules are often involved in these interactions. For example, SH3 and WW domains (conserved regions of approximately 50 and 40 amino acids, respectively) bind proline-rich peptide ligands, and pleckstrin  
5 homology domains (approximately 100 amino acids) interact with both specific phospholipid and protein targets (Pawson, 1995 *supra*).

The Grb7 family represents a family of SH2 domain-containing adaptors which currently contains three members: Grb7, 10 and 14 (Margolis  
10 *et al*, *Proc. Natl. Acad. Sci. USA* 89, 8894-8898, 1992; Stein *et al*, *EMBO J* 13, 1331-1340, 1994; Ooi *et al*, *Oncogene* 10, 1621-1630, 1995; Daly *et al*, *J. Biol. Chem.* 271, 12502-12510, 1996). These proteins share a common overall architecture, consisting of an N-terminal region containing a highly  
15 conserved proline-rich decapeptide motif, a central region harbouring a PH domain and a C-terminal SH2 domain. The central region of approximately 300 amino acids bears significant homology to the *C. elegans* protein mig10, which is required for long range neuronal migration in embryos, otherwise the Grb7 family and mig10 are structurally distinct. However, they exhibit  
20 differences in both SH2 selectivity towards RTKs (Jones *et al*, *J. Biol. Chem.* 272, 8490-8497, 1997) and tissue distribution. The family has therefore evolved to link particular receptors to downstream effectors in a tissue-specific manner. Interestingly, the genes encoding this family appear to have  
25 co-segregated with *ERBB* family genes during evolution. Thus *GRB7*, 10 and 14 are linked to *ERBB2*, *ERBB1* (epidermal growth factor receptor) and *ERBB4*, respectively (Stein *et al* 1994 *supra*; Ooi *et al*, 1995 *supra*; Baker *et al*,  
30 *Genomics* 36, 218-220, 1996). The juxtaposition of *GRB7* and *ERBB2* leads to common co-amplification in human breast cancers, and since the two gene products are functionally linked, likely up-regulation of an undefined *erbB2* signalling pathway. Furthermore, *GRB14* also exhibits differential expression in human breast cancers (Daly *et al*, 1996 *supra*). These two proteins may  
35 therefore modulate RTK signalling in this disease.

In order to identify proteins which bind to this family and therefore identify candidate effectors, we performed a genetic screen using the yeast two hybrid system and Grb14 "bait". This application describes the cloning and characterization of a novel interacting protein, currently designated  
2.2412.

**Disclosure of the Invention:**

Thus, in a first aspect, the present invention provides an isolated polynucleotide molecule encoding a candidate effector protein for the Grb7 family of signalling proteins, wherein the polynucleotide molecule comprises a nucleotide sequence having at least 75% sequence identity to that shown as SEQ ID NO: 1.

Preferably, the polynucleotide molecule comprises a nucleotide sequence having at least 85%, more preferably at least 95%, sequence identity to that shown as SEQ ID NO: 1. Most preferably, the polynucleotide molecule comprises a nucleotide sequence encoding a polypeptide comprising an amino acid sequence substantially corresponding to that shown as SEQ ID NO: 2.

In a preferred embodiment of the invention of the first aspect, the polynucleotide molecule comprises a nucleotide sequence which substantially corresponds to that shown as SEQ ID NO: 1.

The polynucleotide molecule may be a dominant negative mutant which encodes a gene product causing an altered phenotype by, for example, reducing or eliminating the activity of endogenous effector proteins of the Grb7 family of signalling proteins.

The polynucleotide molecule may be incorporated into plasmids or expression vectors (including viral vectors), which may then be introduced into suitable host cells such as bacterial, yeast, insect and mammalian host cells. Such host cells may be used to express the protein encoded by the polynucleotide molecule.

Accordingly, in a second aspect, the present invention provides a host cell transformed with the polynucleotide molecule of the first aspect.

In a third aspect, the present invention provides a method of producing a protein, comprising culturing the host cell of the second aspect under conditions suitable for the expression of the polynucleotide molecule and optionally recovering the protein.

Preferably, the host cell is mammalian or of insect origin. Where the cell is mammalian, it is presently preferred that it be a Chinese hamster ovary (CHO) cell or human embryonic kidney (HEK) 293 cell. Where the host cell is of insect origin, it is presently preferred that it be an insect Sf9 cell.

In a fourth aspect, the present invention provides a purified protein encoded by the polynucleotide molecule of the first aspect.

In a preferred embodiment of this aspect, the purified protein comprises an amino acid sequence substantially corresponding to that shown as SEQ ID NO: 2.

In a fifth aspect, the present invention provides a fusion protein comprising an amino acid sequence substantially corresponding to that shown as SEQ ID NO: 2.

Fusion proteins according to the fifth aspect may include an N-terminal fragment of a protein such as  $\beta$ -galactosidase to assist in the expression and selection of host cells expressing candidate effector protein, or may include a functional fragment of any other suitable protein to confer additional activity(ies).

In a sixth aspect, the present invention provides an antibody or fragment thereof which specifically binds to the protein of the fourth aspect.

The antibody may be monoclonal or polyclonal, however, it is presently preferred that the antibody is a monoclonal antibody. Suitable antibody fragments include Fab, F(ab')<sub>2</sub> and scFv.

In a seventh aspect, the present invention provides an oligonucleotide probe comprising a nucleotide sequence of at least 12 nucleotides, the oligonucleotide probe comprising a nucleotide sequence such that the oligonucleotide probe selectively hybridises to the polynucleotide molecule of the first aspect under high stringency conditions (Sambrook *et al.*, *Molecular Cloning: a Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press).

In a preferred embodiment of this aspect, the oligonucleotide probe is labelled. In a further preferred embodiment of this aspect, the oligonucleotide probe comprises a nucleotide sequence of at least 18 nucleotides.

In an eighth aspect, the present invention provides a method of detecting in a sample the presence of an effector protein for the Grb7 family of proteins, the method comprising reacting the sample with an antibody or fragment thereof the sixth aspect, and detecting the binding of the antibody or fragment thereof.



The method of the eighth aspect may be conducted using any immunoassays well known in the art (e.g. ELISA). The sample may be, for example, a cell lysate or homogenate prepared from a tissue biopsy.

In a ninth aspect, the present invention provides a method of detecting  
5 in a sample the presence of mRNA encoding an effector protein for the Grb7 family of proteins, the method comprising reacting the sample with an oligonucleotide probe of the seventh aspect, and detecting the binding of the probe.

The method of the ninth aspect may be conducted using any  
10 hybridisation assays well known in the art (e.g. Northern blot). The sample may be a poly(A) RNA preparation or homogenate prepared from a tissue biopsy.

Grb7 family proteins exhibit differential expression in certain human cancers (particularly breast and prostate cancer) and may therefore be  
15 involved in tumour progression. Detection of the protein encoded by the cDNA 2.2412 in a sample should provide a useful tumour marker and/or prognostic indicator for these cancers. Furthermore, the interaction of Grb7 family members with 2.2412 may provide a novel target for therapeutic intervention.

20 It is to be understood that methods of detecting suitable agonists and methods of therapy utilising detected agonists also form part of the present invention.

The term "substantially corresponds" as used herein in relation to the nucleotide sequence shown as SEQ ID NO: 1 is intended to encompass minor  
25 variations in the nucleotide sequence which due to degeneracy in the DNA code do not result in a change in the encoded protein. Further, this term is intended to encompass other minor variations in the sequence which may be required to enhance expression in a particular system but in which the variations do not result in a decrease in biological activity of the encoded  
30 protein.

The term "substantially corresponding" as used herein in relation to the amino acid sequences shown as SEQ ID NO: 2 is intended to encompass  
minor variations in the amino acid sequences which do not result in a decrease in biological activity of the protein. These variations may include  
35 conservative amino acid substitutions. The substitutions envisaged are:-

G, A, V, I, L, M: D, E; N, Q; S, T: K, R, H: F, Y, W, H: and

P. N $\alpha$ -alkalamino acids.

The terms "comprise", "comprises" and "comprising" as used throughout the specification are intended to refer to the inclusion of a stated step, component or feature of group of steps, components of features with or without the inclusion of a further step, component or feature or group of steps, components or features.

The invention will hereinafter be described with reference to the accompanying figure and the following, non-limiting example.

#### **Brief description of the accompanying figure:**

Figure 1 provides the nucleotide and amino acid (single letter code) sequence of 2.2412. Numbers refer to distances in base pairs. Ankyrin-type repeat sequences are underlined. An additional repeat sequence is indicated by italics. The stop codon is represented by an asterisk. The original cDNA clone 2.2412 isolated by the two hybrid screen spans nucleotides 694-2664 of this sequence.

Figure 2 provides a map of the 2.2412-binding region on Grb14.

A. Structure of the deletion constructs used in the analysis. Gal4 DNA-BD fusion constructs encoding full length Grb14 (FL), the N-terminal (N), central region (C) and N-terminal + central region (N + C) were generated in the vector pAS2.1.  
B. Results of  $\beta$ -galactosidase activity assays following transformation of the above plasmids into yeast strain Y190 together with the original 2.2412 cDNA clone in pACT-2.

#### **Example: CLONING AND CHARACTERISATION OF 2.2412**

##### **Yeast two hybrid screen**

The yeast two hybrid system exploits protein-protein interactions to reconstitute a functional transcriptional activator which can then be detected using a gene reporter system (Fields and Sternglanz, *TIG*, 10, 286-292, 1994). The technique takes advantage of the properties of the Gal4 protein of the yeast *S. cerevisiae*. The Gal4 DNA binding domain (DNA-BD) or activation domain (AD) alone are incapable of inducing transcription. However, an interaction between two proteins synthesized as DNA-BD- and AD-fusions, respectively, brings the Gal4 domains into close proximity and results in

transcriptional activation of two reporter genes (*HIS3* and *LacZ*) which can be monitored by growth on selective medium and biochemical assays.

A plasmid construct encoding a Gal4 DNA-BD-Grb14 fusion was generated as follows. The plasmid *GRB14/pRcCMVf* containing full length  
5 *GRB14* cDNA (Daly *et al.* 1996) was restricted with *HindIII* and Klenow treated to create blunt ends. and then digested with *BclI* to release three fragments of approximately 1.1, 4.2 and 1.7 kb. The 1.7 kb fragment was isolated and cloned into the *NdeI* (Klenow treated) and *BamHI* sites of the yeast expression vector pAS2.1 (Clontech) to generate *GRB14/pAS2.1*  
10 containing an in-frame fusion of full length Grb14 with the GAL4 DNA-BD. This construct was introduced by electroporation into the yeast strain CG1945 (*MATa*, *ura3-52*, *his3-200*, *ade2-101*, *lys2-801*, *trp1-901*, *leu2-3*, *112*, *gal4-542*, *gal80-538*, *cyh<sup>r</sup>2*. *LYS2::GAL1 UAS-GAL1 TATA-HIS3*, *URA3::GAL4<sup>17mers</sup>(x3)-CYC1 TATA-lacZ*) selecting for tryptophan  
15 prototrophy. The expression of the fusion protein was verified by Western blot analysis with antibodies directed against the Flag epitope and the Gal4 DNA-BD. The recipient strain was then grown to mid-log phase and a human liver cDNA library in the vector pACT2 (Clontech) introduced using the LiAc procedure (Schiestl and Gietz, *Curr. Genet.* 16. 339-346. 1989). Transformants  
20 were then selected for tryptophan, leucine and histidine prototrophy in the presence of 5mM 3-aminotriazole.

From a screen of  $1 \times 10^6$  clones, 39 colonies were initially selected on synthetic complete (SC)-leu-his-trp + 3AT medium and were then tested for  $\beta$ -galactosidase activity. 12 clones scored positive in the latter assay and were  
25 subjected to cycloheximide (CHX) curing to remove the bait plasmid by streaking out on SC-leu media containing 10ug/ml CHX (pAS2-1 contains the *CYH2* gene which restores CHX sensitivity to CG1945 cells). This enabled confirmation of the bait dependency of *LacZ* activation and subsequent isolation of the pACT2 plasmids encoding interacting proteins by standard  
30 methodology (Philippsen *et al.* *Methods in Enzymology* 194, 170-177). Back transformations were then performed in which these pACT2 plasmids were introduced into CG1945 strains containing the bait plasmid (*GRB14/pAS2-1*) or constructs encoding non-related Gal4 DNA-BD fusions in order to confirm the specificity of the interactions.

35 The DNA sequences of the cDNA inserts were then obtained by cycle sequencing (f-mol kit, Promega) using pACT2-specific and/or clone-specific

primers. Based on their nucleotide sequences the 12 interacting clones were classified into 6 independent groups (see Table I).

**TABLE I: Characterization of cDNA clones isolated by the yeast two hybrid screen.**

	Class	No. of clones	Identity	Mean RLU (Liquid assay)	Colour intensity (Filter assay)
10	1	6	Nedd4	$2.86 \times 10^6$	++++
	2	2	Htk	$1.86 \times 10^5$	++
	3	1	2.2412	$5.18 \times 10^6$	++++
	4	1	Proteosome	$3.88 \times 10^2$	+/-
	5	1	Somatostatin receptor	$1.45 \times 10^3$	+/-
15	6	1	L-arginine:glycine amidinotransferase	$8.61 \times 10^2$	+/-

The 12 clones exhibiting activation of both the *HIS3* and *lacZ* reporter genes were divided into 6 groups by sequence analysis of their cDNA inserts. Results of  $\beta$ -galactosidase activity assays performed using two methodologies are shown. The liquid culture-derived method (Galacto-Light, TROPIX) is more quantitative; results are given in mean relative light units (RLU) and are normalized for the protein content of the samples. Blue/white screening of the cDNA clones was also performed using a colony lift filter assay (Clontech). The intensity of blue colour development over approximately 2h is scored from +/- (very weak) to ++++ (strong).

Six clones were partial cDNAs corresponding to Nedd4, a multidomain protein containing a calcium-dependent phospholipid binding (CaLB) domain, four WW domains and a C-terminal region homologous to the E6-AP carboxyl-terminus (Kumar *et al.* *Biochem. Biophys. Res. Commun.* 185, 1155-1161, 1992; Sudol *et al.* *J. Biol. Chem.* 270, 14733-14741, 1995; Huibregtse *et al.* *Proc. Natl. Acad. Sci. USA* 92, 2563-2567, 1995). The latter is likely to confer E3 ubiquitin-protein ligase activity on Nedd4. The pACT2 clones isolated encoded the CaLB domain together with the first 22 amino acids of the first WW domain.

Two clones encoded the intracellular region and part of the extracellular domain of Htk, which is a RTK of the Eph family (Bennett *et al* *J. Biol. Chem.* 269, 14211-14218, 1994). The recruitment of Grb14 by Htk is of interest for two reasons. First, the expression profile of both Htk and the murine homologue myk-1 are indicative of a potential role in mammary gland development and neoplasia (Andres *et al* *Oncogene* 9, 1461-1467, 1994; Berclaz *et al* *Biochem. Biophys. Res. Comm.* 226, 869-875, 1996). Second, Eph family members may be involved in the regulation of cell migration (Tessier-Lavigne, *Cell* 82, 345-348, 1995), which is intriguing given the homology of the Grb7 family to the *C. elegans* protein mig10 (Stein *et al.* 1994 *supra*).

A novel cDNA of 1971 bp, designated 2.2412, was also isolated. This clone encoded a polypeptide of 657 amino acids in frame with the Gal4 DNA-BD. The cDNA did not contain a stop codon, and this, together with the Northern analysis described below, indicated that it was incomplete. This DNA fragment was therefore used as a probe to screen a human placental cDNA library (5' STRETCH PLUS, Clontech, in  $\lambda$ gt10). This resulted in the isolation of two clones, designated clone 8 and clone 12. Clone 8 was approximately 2 kb and overlapped the original 2.2412 clone by 900 bp at the 3' end. This clone provided the carboxy-terminal end of the 2.2412 protein sequence (Figure 1). Clone 12 was approximately 3.5 kb and to date has provided an additional 692 bp of sequence information in the 5' direction. The nucleotide and protein sequence for 2.2412 provided by these overlapping clones is shown in Figure 1. Since a 5' initiation codon has yet to be identified the coding sequence still appears to be incomplete.

#### Further characterization of 2.2412

Database searches using the 2.2412 cDNA sequence revealed significant homology with a large number of proteins containing ankyrin-like repeats. These sequences were first identified as homologous regions between certain cell cycle regulatory proteins and the *Drosophila* protein Notch (Breedon and Nasmyth, *Nature* 329, 651-654, 1987) but subsequently they have been identified in a wide variety of other proteins where they are thought to function in protein-protein interactions (Bork, *Proteins* 17, 363-374, 1993). Subsequent analysis of the protein sequence identified 18 consecutive ankyrin repeats and an additional repetitive element (Figure 1). The ankyrin repeat region is followed by a stretch of approximately 40 amino

acids rich in serine residues. The remaining C-terminal region has a relatively high content of charged amino acids.

#### Northern analysis of 2.2412 mRNA expression

5 Northern blot analysis of multiple tissue northern (Clontech) was performed using the original 2.2412 cDNA as a probe. This resulted in the detection of a single mRNA transcript of approximately 7 kb in all tissues examined with the exception of the kidney. Expression was particularly high in skeletal muscle and placenta. The size of this transcript compared to that  
10 of the 2.2412 clone indicates that the latter represents only a partial cDNA.

#### Genomic localization of the 2.2412 gene

Fluorescence *in situ* hybridization of the original 2.2412 cDNA to normal metaphases (Baker *et al.* 1996 *supra*) and reference to the FRA10A  
15 fragile site at 10q23.32 localized the gene to between chromosome 10q23.2 and proximal 10q23.32. Interestingly, deletions in the 10q22-25 region of chromosome 10 have been detected in a variety of human cancers including breast, prostate, renal, small cell lung and endometrial carcinomas, glioblastoma multiforme, melanoma and meningiomas, suggesting the  
20 presence of one or more tumour suppressive loci in this region (Li *et al.*, *Science* 275, 1943-1947, 1997; Steck *et al.*, *Nature Genetics* 15, 356-362, 1997, and references therein). Two candidate tumour suppressor genes have been identified in this region (MMAC1/PTEN and MXI1, Li *et al.* 1997 *supra*; Steck *et al.* 1997 *supra*; Albarosa *et al.*, *Hum. Genet.* 95, 709-711, 1995).

25

#### Analysis of the interaction between 2.2412 and Grb7 family members

cdNAs encoding the full length and N- and C-terminal regions of the original 2.2412 cDNA clone (nucleotides 694-2664, 694-1614 and 1615-2664 of the sequence shown in Figure 1, respectively) were cloned into the vector  
30 pGEX4T2 (Pharmacia). The full length construct was generated by subcloning from the pACT2 clone as a NdeI fragment, whereas the shorter constructs were synthesized by directional cloning of PCR products. The corresponding GST-fusion proteins were purified from IPTG-induced bacterial cultures using glutathione-agarose beads (Smith and Johnson, *Gene*  
35 67, 31-40, 1988). These immobilized fusion proteins were then incubated with lysates from cells expressing Flag epitope-tagged Grb14 (Daly *et al.* 1996

*supra*) or human breast cancer cells expressing high levels of Grb7 (SK-BR-3: Stein *et al.* 1994) as described previously (Daly *et al.* 1996). Following washing, bound proteins were detected by Western blot analysis. The results indicated that 2.2412 bound specifically to both Grb14 and Grb7 *in vitro*, and that the N-terminal fusion protein bound more strongly than that derived from the C-terminus. These data, obtained using a different methodology for detecting protein-protein interactions to the yeast two hybrid system, confirm that 2.2412 interacts with Grb14. Furthermore, 2.2412 also binds Grb7. Consequently 2.2412 appears to represent a general effector for the Grb7 family.

#### Mapping of the 2.2412 binding region on Grb14

In order to identify the region of Grb14 that interacts with 2.2412, a series of Grb14 deletion mutants were generated by cloning PCR fragments synthesized using the appropriate flanking primers into the vector pAS2.1. These fragments spanned the following regions: N-terminus ("N", amino acids 1-110), the central region ("C") encompassing the mig10 homology and the "between PH and SH2" (BPS) domain (amino acids 110-437) and the N-terminal and central regions ("N + C", amino acids 1-437). These plasmids were individually transformed into the yeast strain Y190 (*MATa, ura3-52, his3-200, ade2-101, lys2-801, trp1-901, leu2-3, 112, gal4Δ, gal80Δ, cyh<sup>r</sup>2, LYS2::GAL1UAS-HIS3TATA-HIS3, URA3::GAL1UAS-GAL1TATA-lacZ*) and expression of the appropriately sized Gal4 DNA-BD fusion proteins confirmed by Western blotting. Following transformation of the resulting yeast strains with the original 2.2412 cDNA clone in pACT-2, the strength of the interaction was determined by either liquid- or filter-based  $\beta$ -galactosidase assays. The results are presented in Figure 2, and demonstrate that the N-terminal region of Grb14 is not only required, but is also sufficient, for binding 2.2412. This supports the hypothesis that 2.2412 represents a general effector for the Grb7 family, since the N-terminal region of these proteins contains a highly conserved proline-rich motif which may mediate this interaction.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.



Sequence listings:

## SEQUENCE LISTING

Applicant: Garvan Institute of Medical Research

Title of Invention: A potential effector for the Grb7 family of signalling proteins.

Current Application Number:

Current Filing Date:

Prior Application Number: P09388

Prior Application Filing Date: 1997-09-23

Number of ID SEQ Nos: 2

Software: PatentIn Ver. 2.0

SEQ ID NO: 1

Length: 3400

Type: DNA

Organism: Homo sapiens

Sequence: 1

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attcctcttc ataatgcgat cctcttttgg catgctgaag tagtcaatct ccttttgcga 60
catgggtgcag accccaatgc tcgagataat tgggaattata ctccctctca tgaagctgca 120
attaagagaa agattgatgt ttgcattgtg ctgttacagc atggagctga gccaaccatc 180
cgaaatacag atgaagagac agcattggat tttagcagat catctgccaa agcagtgctt 240
actggtgaa ataaagaaga tgaactctta gaaagtgcc aaggtggcaa tgaagaaaaa 300
atgatggctc tactcacacc attaaatgtc aactgccacg caagtgatgg cagaagtcca 360
actccattac atttggcagc aggatataac agagtaaaag ttgtacagct gttactgcaa 420
catggacgtg atgtccatgc taaagataaa ggtgatctgg taccattaca caatgcctgt 480
tcttatggtc attatgaagt aactgaactt ttggccaagc atggtggctg tgaatatgca 540
atggacttgt ggcaattcac tctctctcat gaggcagctt ctaagaacag ggttgaagta 600
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gctatagact tggctccac accacagtta aagaagaagt tagcatatga atttaaaagg 720
cactcgattg tgaagctgc acgagaagct gatgttactc gaatcaaaaa acatctctct 780
ctggaattgg tgaatttcaa gcatcctcaa acacatgaaa cagcatlga tttgtctgct 840
gcactcccat atcccaaaa aagaacaaata tgtgaactgt tgctaagaaa aggagcacaac 900
atcaatgaaa agactaaaga attcttgact cctctgcacg tggcatctga gaaagctcat 960
aatgatgttg ttgaagtagt ggtgaaacat gaagcaaaag ttaatgctct ggataatctt 1020
ggtcagactt cctcacagc agctgcatat ttggtcatc tacaacacct cgcgcctactc 1080
ctgagctatg ggtgtgatcc taacattata tcccttcagg gctttactgc ttacagatg 1140
ggaaatgaaa atgtacagca actcctccaa gagggtatct cattaggtaa ttcagaggca 1200
gcagacaaat tgtctgaactg tccaaggctt ggagatgtcg aaactgttaa aaaacttgtt 1260
actgttcaga gtgtcaactg cagagacatt gaaggcgctc agtctacacc acttcatctt 1320
cgagctgggt ataacagagt gtccgtggtg gaatatctgc tacagcatag agctgatgtg 1380
catgctaaag ataaaggagg ccttgtacct ttgcacaatg catgttcttfa cggagcattat 1440
gaagttgcag aactcttgtt taaacatgga cgactagtta atgtagctga tttatggaaa 1500
tttacacctt tacatgaagc agcagcaaaa ggaaaatatg aaatttgcna acttctgctc 1560
cagcatgttg cagacccatc aaaaaaaac agggatggaa atactccttt ggaacttgtt 1620
aaagatggag atacagatct tcaagatctg cttagggggg atgcagcttt gctagatgct 1680
gccaaagaag gtgtgtttagc cagagtgtaag aagtgtgtct ctctgtatna tgttaattgc 1740
cgcgataccc aaggcagaca ttcaacacct ttacatttag cagctgtgta taataattta 1800
gaagtgcagc agtatttgtt acaacacgga gctgatgtga atgcccaaga caaaggagga 1860
cttattcctt tacataatgc agcatcttac gggcatgtag atgtagcagc tctactaata 1920

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aagtataatg catctctcaa tgcacggac aaatgggctt tccacacctt gcacgaagca 1980
gcccaaaagg gacgaacaca gctttgtgct ttgttgctag cccatggagc tgarcggact 2040
cttaaaaatc aggaaggaca aacaccttta gatttagttt cagcagatga tgtcagcgct 2100
ctctgcagac cagccatgcc cccatctgct ctgccccttt gttacaaagc tcaagtgctc 2160
aatgggtgta gaagcccgag agccactgca gatgctctct ctccaggtrc atctagccca 2220
tcaagctctt ctgcagccag cagtcttgac aacttatctg ggagtttttc agaactgtct 2280
tcagtagtta gttcagtggt aacagagggt gcttccagtt tggagaaaaa ggaggtttca 2340
ggagtagatt ttacataaac tcaattcgta aggaatcttg gacttgagca cctaattggt 2400
atatttgaga gagaacagat cactttggat gtatttagtt agatggggga caaggagctg 2460
aaggagattg gaatcaatgc ttatggacat aggcacaaac taattaaagg agtcgagaga 2520
cttatctccg gacaacaagg tcttaaccca tatttaacct tgaacaacct tggtagtgga 2580
acaattctta tagatctgtc tcttgatgat aaagagtttc agtctgtgga ggaagagatg 2640
caaaagtacag ttcgagagca cagagatgga ggtcatgcag gtggaaatct gtgcagatac 2700
aataattcta agattcagaa ggtttgtaac aagaaactat gggaaagata cactcaccgg 2760
agaaaagaag tttctgaaga aaaccacaac catgccaatg aacgaatgct atttcatggg 2820
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ggtagttttg gagctggcat ttattttgct gaaaactctt ccaaaagcaa tcaatagta 2940
tatggaattg gaggaggtac tgggtgtcca gtccacaag acagatcttg ttacatttgc 3000
cacaggcagc tgcctctttg ccgggtaacc ttgggaaagt ctttccctga gttcagtgca 3060
atgaaaatgg cacattctcc tccaggtcat cactcagtca ctggtaagrc cagtgtaaat 3120
ggcctagcat tagctgaata tgttatttac agaggagaac agccttatrc tgagtattta 3180
attactacc agattatgag gctgaaggt atggctgagt gataaataat tatttttaaga 3240
aactaattcc actgaacctc aaatcatcaa agcagcagtg gcctctatgt ttactacctt 3300
tgctgaaaaa aatatcatct gccccagggc ctgtggcana aggataaaaa tgtgaacgaa 3360
gtttaaactt ctgacttgat aaagctttaa taatgtacag 3400

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SEQ ID NO: 2  
 Length: 1074  
 Type: PRT  
 Organism: Homo sapiens

Sequence: 2

Ile Pro Leu His Asn Ala Cys Ser Phe Gly His Ala Glu Val Val Asn  
 1 5 10 15

Leu Leu Leu Arg His Gly Ala Asp Pro Asn Ala Arg Asp Asn Thr Asn  
 20 25 30

Tyr Thr Pro Leu His Glu Ala Ala Ile Lys Gly Lys Ile Asp Val Cys  
 35 40 45

Ile Val Leu Leu Gln His Gly Ala Glu Pro Thr Ile Arg Asn Thr Asp  
 50 55 60

Gly Arg Thr Ala Leu Asp Leu Ala Asp Pro Ser Ala Lys Ala Leu  
 65 70 75 80

Thr Gly Glu Tyr Lys Lys Asp Glu Leu Leu Glu Ser Ala Arg Ser Gly  
 85 90 95

Asn Glu Glu Lys Met Met Ala Leu Leu Thr Pro Leu Asn Val Asn Cys  
 100 105 110

His Ala Ser Asp Gly Arg Lys Ser Thr Pro Leu His Leu Ala Ala Gly  
 115 120 125

Tyr Asn Arg Val Lys Ile Val Gln Leu Leu Leu Gln His Gly Arg Asp  
 130 135 140

Val His Ala Lys Asp Lys Gly Asp Leu Val Pro Leu His Asn Ala Cys

15

145		150		155		160
Ser Tyr Gly His Tyr Glu Val Thr Glu Leu Leu Val Lys His Gly Gly	165		170		175	
Cys Val Asn Ala Met Asp Leu Trp Gln Phe Thr Pro Leu His Glu Ala	180		185		190	
Ala Ser Lys Asn Arg Val Glu Val Cys Ser Leu Leu Ser Tyr Gly	195		200		205	
Ala Asp Pro Thr Leu Leu Asn Cys Lys Asn Lys Ser Ala Ile Asp Leu	210		215		220	
Ala Pro Thr Pro Gln Leu Lys Glu Arg Leu Ala Tyr Glu Phe Lys Gly	225		230		235	
His Ser Leu Leu Gln Ala Ala Arg Glu Ala Asp Val Thr Arg Ile Lys	245		250		255	
Lys His Leu Ser Leu Glu Met Val Asn Phe Lys His Pro Gln Thr His	260		265		270	
Glu Thr Ala Leu His Cys Ala Ala Ala Ser Pro Tyr Pro Lys Arg Lys	275		280		285	
Gln Ile Cys Glu Leu Leu Leu Arg Lys Gly Ala Asn Ile Asn Glu Lys	290		295		300	
Thr Lys Glu Phe Leu Thr Pro Leu His Val Ala Ser Glu Lys Ala His	305		310		315	
Asn Asp Val Val Glu Val Val Val Lys His Glu Ala Lys Val Asn Ala	325		330		335	
Leu Asp Asn Leu Gly Gln Thr Ser Leu His Arg Ala Ala Tyr Cys Gly	340		345		350	
His Leu Gln Thr Cys Arg Leu Leu Leu Ser Tyr Gly Cys Asp Pro Asn	355		360		365	
Ile Ile Ser Leu Gln Gly Phe Thr Ala Leu Gln Met Gly Asn Glu Asn	370		375		380	
Val Gln Gln Leu Leu Gln Glu Gly Ile Ser Leu Gly Asn Ser Glu Ala	385		390		395	
Asp Arg Gln Leu Leu Glu Ala Ala Lys Ala Gly Asp Val Glu Thr Val	405		410		415	
Lys Lys Leu Cys Thr Val Gln Ser Val Asn Cys Arg Asp Ile Glu Gly	420		425		430	
Arg Gln Ser Thr Pro Leu His Phe Ala Ala Gly Tyr Asn Arg Val Ser	435		440		445	
Val Val Glu Tyr Leu Leu Gln His Gly Ala Asp Val His Ala Lys Asp	450		455		460	
Lys Gly Gly Leu Val Pro Leu His Asn Ala Cys Ser Tyr Gly His Tyr						

465	470								475								480						
Glu Val Ala Glu Leu Leu Val Lys His	Gly Ala Val Val Asn Val Ala																						
485								490								495							
Asp Leu Trp Lys Phe Thr Pro Leu His	Glu Ala Ala Ala Lys Gly Lys																						
500								505								510							
Tyr Glu Ile Cys Lys Leu Leu Leu Gln His	Gly Ala Asp Pro Thr Lys																						
515								520								525							
Lys Asn Arg Asp Gly Asn Thr Pro Leu Asp	Leu Val Lys Asp Gly Asp																						
530								535								540							
Thr Asp Ile Gln Asp Leu Leu Arg Gly Asp	Ala Leu Leu Asp Ala																						
545								550								555							
Ala Lys Lys Gly Cys Leu Ala Arg Val Lys	Lys Leu Ser Ser Pro Asp																						
565								570								575							
Asn Val Asn Cys Arg Asp Thr Gln Gly Arg	His Ser Thr Pro Leu His																						
580								585								590							
Leu Ala Ala Gly Tyr Asn Asn Leu Glu Val	Ala Glu Tyr Leu Leu Gln																						
595								600								605							
His Gly Ala Asp Val Asn Ala Gln Asp Lys	Gly Gly Leu Ile Pro Leu																						
610								615								620							
His Asn Ala Ala Ser Tyr Gly His Val Asp	Val Ala Ala Leu Leu Ile																						
625								630								635							
Lys Tyr Asn Ala Ser Leu Asn Ala Thr Asp	Lys Trp Ala Phe Thr Pro																						
645								650								655							
Leu His Glu Ala Ala Gln Lys Gly Arg Thr	Gln Leu Cys Ala Leu Leu																						
660								665								670							
Leu Ala His Gly Ala Asp Pro Thr Leu Lys	Asn Gln Glu Gly Gln Thr																						
675								680								685							
Pro Leu Asp Leu Val Ser Ala Asp Asp Val	Ser Ala Leu Leu Thr Ala																						
690								695								700							
Ala Met Pro Pro Ser Ala Leu Pro Ser Cys	Tyr Lys Pro Gln Val Leu																						
705								710								715							
Asn Gly Val Arg Ser Pro Gly Ala Thr Ala	Asp Ala Leu Ser Ser Gly																						
725								730								735							
Pro Ser Ser Pro Ser Ser Leu Ser Ala Ala	Ser Ser Leu Asp Asn Leu																						
740								745								750							
Ser Gly Ser Phe Ser Glu Leu Ser Ser Val	Val Ser Ser Ser Gly Thr																						
755								760								765							
Glu Gly Ala Ser Ser Leu Glu Lys Lys Glu	Val Pro Gly Val Asp Phe																						
770								775								780							
Ser Ile Thr Gln Phe Val Arg Asn Leu Gly	Leu Glu His Leu Met Asp																						
785								790								795							

785				790				795				800				
Ile	Phe	Glu	Arg	Glu	Gln	Ile	Thr	Leu	Asp	Val	Leu	Val	Glu	Met	Gly	
				805					810					815		
His	Lys	Glu	Leu	Lys	Glu	Ile	Gly	Ile	Asn	Ala	Tyr	Gly	His	Arg	His	
				820					825					830		
Lys	Leu	Ile	Lys	Gly	Val	Glu	Arg	Leu	Ile	Ser	Gly	Gln	Gln	Gly	Leu	
				835					840					845		
Asn	Pro	Tyr	Leu	Thr	Leu	Asn	Thr	Ser	Gly	Ser	Gly	Thr	Ile	Leu	Ile	
				850					855					860		
Asp	Leu	Ser	Pro	Asp	Asp	Lys	Glu	Phe	Gln	Ser	Val	Glu	Glu	Glu	Met	
				865					870					875		
Gln	Ser	Thr	Val	Arg	Glu	His	Arg	Asp	Gly	Gly	His	Ala	Gly	Gly	Ile	
				885					890					895		
Phe	Asn	Arg	Tyr	Asn	Ile	Leu	Lys	Ile	Gln	Lys	Val	Cys	Asn	Lys	Lys	
				900					905					910		
Leu	Trp	Glu	Arg	Tyr	Thr	His	Arg	Arg	Lys	Glu	Val	Ser	Glu	Glu	Asn	
				915					920					925		
His	Asn	His	Ala	Asn	Glu	Arg	Met	Leu	Phe	His	Gly	Ser	Pro	Phe	Val	
				930					935					940		
Asn	Ala	Ile	Ile	His	Lys	Gly	Phe	Asp	Glu	Arg	His	Ala	Tyr	Ile	Gly	
				945					950					955		
Gly	Met	Phe	Gly	Ala	Gly	Ile	Tyr	Phe	Ala	Glu	Asn	Ser	Ser	Lys	Ser	
				965					970					975		
Asn	Gln	Tyr	Val	Tyr	Gly	Ile	Gly	Gly	Gly	Thr	Gly	Cys	Pro	Val	His	
				980					985					990		
Lys	Asp	Arg	Ser	Cys	Tyr	Ile	Cys	His	Arg	Gln	Leu	Leu	Phe	Cys	Arg	
				995					1000					1005		
Val	Thr	Leu	Gly	Lys	Ser	Phe	Leu	Gln	Phe	Ser	Ala	Met	Lys	Met	Ala	
				1010					1015					1020		
His	Ser	Pro	Pro	Gly	His	His	Ser	Val	Thr	Gly	Arg	Pro	Ser	Val	Asn	
				1025					1030					1035		
Gly	Leu	Ala	Leu	Ala	Glu	Tyr	Val	Ile	Tyr	Arg	Gly	Glu	Gln	Ala	Tyr	
				1045					1050					1055		
Pro	Glu	Tyr	Leu	Ile	Thr	Tyr	Gln	Ile	Met	Arg	Pro	Glu	Gly	Met	Val	
				1060					1065					1070		
Asp	Gly															

**Claims:**

1. An isolated polynucleotide molecule encoding a candidate effector protein for the Grb7 family of signalling proteins, wherein the polynucleotide molecule comprises a nucleotide sequence having at least 75% sequence identity to that shown as SEQ ID NO: 1.
2. A polynucleotide molecule according to claim 1, wherein the polynucleotide molecule comprises a nucleotide sequence having at least 85% sequence identity to that shown as SEQ ID NO: 1.
3. A polynucleotide molecule according to claim 1, wherein the polynucleotide molecule comprises a nucleotide sequence having at least 95% sequence identity to that shown as SEQ ID NO: 1.
4. A polynucleotide molecule according to claim 1, wherein the polynucleotide molecule comprises a nucleotide sequence which substantially corresponds to that shown as SEQ ID NO: 1.
5. A host cell transformed with a polynucleotide molecule according to any one of the preceding claims.
6. A host cell according to claim 5, wherein the host cell is a mammalian, insect, yeast or bacterial host cell.
7. A method of producing a protein, comprising culturing the host cell of claim 5 or 6 under conditions suitable for the expression of the polynucleotide molecule and optionally recovering the protein.
8. A purified protein encoded by a polynucleotide molecule according to any one of claims 1 to 4.
9. A purified protein according to claim 8, wherein the protein comprises an amino acid sequence substantially corresponding to that shown as SEQ ID NO: 2.

10. A fusion protein comprising an amino acid sequence substantially corresponding to that shown as SEQ ID NO: 2.
11. An antibody or fragment thereof which specifically binds to a protein  
5 according to claim 8 or 9.
12. An oligonucleotide probe comprising a nucleotide sequence of at least 12 nucleotides, the oligonucleotide probe comprising a nucleotide sequence such that the oligonucleotide probe selectively hybridises to the  
10 polynucleotide molecule of any one of claims 1 to 4 under high stringency conditions.
13. An oligonucleotide probe according to claim 12, wherein the oligonucleotide probe comprises a nucleotide sequence of at least 18  
15 nucleotides.
14. A method of detecting in a sample the presence of an effector protein for the Grb7 family of proteins, the method comprising reacting the sample with an antibody or fragment thereof according to claim 11.  
20
15. A method of detecting in a sample the presence of mRNA encoding an effector protein for the Grb7 family of proteins, the method comprising reacting the sample with an oligonucleotide probe of claim 12 or 13.

[illegible]



GGATCTTGTTAAAGATGGAGATACAGATATTCAGATCTGCTTAGGGGAGATGCAGCTTGCTAGATGCT 1680  
D L V K D G D T D I Q D L L R G D A A L L D A

GCCAGAGAGGGTGTGTTAGCCAGAGTGAAGAAGTTGTCTTCTCTGATATGTAAATTCGCCGGATACCC 1750  
A K K G C L A R V K K L S S P D N V N C R D T

AAGCGAGACATTCAACACCTTTACATTTAGCAGCTGGTTATATAATTTAGAAGTTGCAGAGTATTTGTT 1820  
Q G R H S T P L H L A A G Y N N N L E V A E Y L L

ACAAACCGGAGCTGATGTAATGCCAAGACAAGGAGGACTTATTCCTTACATAATGCAGCATCTTAC 1890  
Q H G A D V N A Q D K G G L I P L H N A A S Y

GGGCATGTAGATGTAGCAGCTCTACTAATAAAGTATAATGCATCTCTCAATGCCACGGACAAATGGGCTT 1960  
G H V D V A A L L I K Y N A S L N A T D K W A

TCACACCTTTGCACGAAGCAGCCAAAAGGGACGAACACAGCTTTGTGCTTTGTTGCTAGCCATGGAGC 2030  
F T P L H E A A Q K G R T Q L C A L L L A H G A

TGACCCAGACTCTAAAAATCAGGAAGGACAAACCTTTAGATTAGTTTCAGCAGATGATGTCAGCGCT 2100  
D P T L K N Q E G Q T P L D L V S A D D V S A

CTTCTGACAGCAGCATGCCCCCATCTGCTCTGCGCTCTTGTGTTACAAGCTCAAGTCTCAATGGTGTGA 2170  
L L T A A M P P S A L P S C Y K P Q V L N G V

GAAAGCCAGGAGCCACTGTCAGATGCTCTCTCTCAGGTCCATCTAGCCCATCAAGCCTTTCTGCAGCCAG 2240  
R S P G A T A D A L S S G P S S P S L S A A S

CAGTCTTGACAACITATCTGGGAGTTTTTCAGAAGTCTCTCAGTAGTTAGTTCAGTGGAAACAGAGGCT 2310  
S L D N L S G S F S E L S S V V S S G T E G

GCTTCCAGTTTGGAGAAAAGGAGGTTCTCAGGAGTAGATTAGCATAACTCAATTCTGTAAGGAATCTTG 2380  
A S S L E K K E V P G V D F S I T Q F V R N L

GACTTGAGCACCTAATGGATATATTTGAGAGAGAACAGATCACTTTGGATGTATAGTTGAGATGGGCA 2450  
G L E H L M D I F E R E Q I T L D V L V E M G H

CAAGGAGCTGAAGGAGATTGGAATCAATGCTTATGGACATAGGCACAACTAATTAAGAGAGTCGAGAGA 2520  
K E L K E I G I N A Y G H R H K L I K G V E R

CTTATCTCCGGACAACAGGCTTAACCCATATTTAACTTTGAACACCTCTGGTAGTGGAACTATTTTA 2590  
L I S G Q Q G L N P Y L T L N T S G S G T I L

TAGATCTGCTCCTGATGATAAGAGTTTCAGTCTGTGGAGGAGAGATGCAAGTACAGTTTCGAGAGCA 2660  
I D L S P D D K E F Q S V E E E M Q S T V R E H

CAGAGATGGAGGTCATGCAGGTGGAATCTTCAACAGATACAAATTTCTCAAGATTCGAAGGTTTGTAA 2730  
R D G G H A G G I F N R Y N I L K I O K V C N

AAGAACTATGGGAAGATACACTCACCGAGAAAAGAAGTTCTGGAAGAAACACACAACTGCCAATG 2800  
K K L W G G Y T H R R K E V S E E N H H A N

AACGAATGCTATTTCATGGGTTCTCTTTGTGAATGCAATTATCCACAAAGGCTTGTGAAGAGGCAATG 2870  
E R M L F H G S P F V N A I I H K G F D E R H A

GTACATAGGTGGTATGTTTGGAGCTGGCATTATTTTGTGAAAACCTCTTCCAAAAGCAATCAATATGTA 2940  
Y I G G M F G A G I Y F A E N S S K S N Q Y V

TATGGAATTGGAGGAGTACTGGGTCTCCAGTTCCAAAAGACAGATCTTGTACATTTGCCACAGGCAGC 3010  
Y G I G G T G C P V H K D R S C Y I C H R Q

TGCTCTTTTCCGGGTAACTTGGGAAAGTCTTCTCTGAGTTCAGTGCATGAAATGGCAATCTTCTCC 3080  
L L F C R V T L G K S F L Q F S A M K M A H S P

TCAGGTCATCATGCTAGTGGTGGCCAGTGTAAATGGCCCTAGCATTAGCTGAATATGTTATTATC 3150  
P G H H S V T G R P S V N G L A L A E Y V I Y

AGAGGAGAACAGGCTTATCTGAGTATTTAATTAATCTTACCAGATATGAGGCTCAAGGATTTGGTCGATG 3220  
R G E Q A Y P E Y L I T Y Q M R P E G M V D

GATAAATAGTTATTTTAAAGAACTAATTCACCTGAACCTAAAAATCATCAAGCAGCAGTGGCCCTACGT 3290  
G

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TTTACTCCTTTGCTGAAAAAAAAATCATCTTGCCACAGGCTGTGGCAAAGGATAAAATGTGAACGAA 3360

GTTTAACATTCTGACTTGATAAAGCTTTAATAATGTACAG

09509196-022070

4/4

A

CONSTRUCT

STRUCTURE

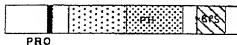
N



C



N + C



FL



B

CONSTRUCT

 MEAN RLU  
 (LIQUID ASSAY)  
 ( $\times 10^3$ )

 COLOUR INTENSITY  
 (FILTER ASSAY)

pAS2.1

4

-

N

109

++

C

3

-

N + C

194

++

FL

242

+++

FIGURE 2

# DECLARATION, POWER OF ATTORNEY AND PETITION

As a below named inventor, I hereby declare that:

My residence, post office and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original first and joint inventor (if plural names are listed below) of the subject matter claimed and for which a patent is sought on the invention entitled:

## **A POTENTIAL EFFECTOR FOR THE GRB7 FAMILY OF SIGNALLING PROTEINS**

the specification of which

☐ is attached hereto ☒ was filed on **23 September 1998** as Application No. **PCT/AU98/00795** and was amended on (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56(a)

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed	
PO 9388	Australia	23 September 1997	<input checked="" type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>
[Number]	[Country]	[Day/Month/Year Filed]	Yes	No

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

[Application Serial no]	[Filing Date]	[Status: patented, pending, abandoned]

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issued thereon.

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Columbia Square  
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Washington, District of Columbia, 20004  
United States of America

with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and all future correspondence should be addressed to them.

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./2

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